International Journal of Pharmaceutics, 81 (1992) 153–167

IJP 02696

Evaluation of heat-conduction microcalorimetry in pharmaceutical stability studies. V. A new approach for continuous measurements in abundant water vapour

M. Angberg [1], C. Nyström [1] and S. Castensson [2]

[1] Department of Pharmaceutics, Uppsala University and [2] Kabi Pharmacia AB, Uppsala (Sweden)

(Received 6 September 1991)
(Accepted 18 October 1991)

Key words: Microcalorimetry; DSC; Anhydrous lactose; Water; Hydration; Stability

Summary

A technique which allows continuous microcalorimetric measurement in abundant water vapour at 100% relative humidity (RH) has been designed and evaluated at 25.0°C. The incorporation of water of hydration in various types of anhydrous lactose powder was studied. In some experiments, lactose samples were prestored at humidities ranging from 58 to 94% RH and thereafter characterized with the new technique at 100% RH. The incorporation process is the main contributor of heat. The heat contributions from other sources in the sample vessel were also investigated. Heats of adsorption and vaporization were negligible, since they cancelled each other out. However, the heat from the incorporation process was somewhat reduced by the heat of mutarotation from β- to α-lactose and possibly also by the heat from a minute amount of dissolution of the lactose. The enthalpy change for the bonding of water of hydration to roller-dried β-lactose was calculated as approx. 16 kJ/mol at 25°C.

Introduction

Heat-conduction microcalorimetry can continuously measure the rate of heat change in a sample, which is ideally proportional to the rate of the process. Microcalorimetry has therefore been used in stability measurements (Angberg et al., 1988; Hansen et al., 1989; Pikal and Dellerman, 1989; Angberg et al., 1990; Oliyai and Lindenbaum, 1991). To be able to perform another type of stability study it was desirable to have access to a microcalorimetric measurement technique which exposes a solid sample to a constant relative humidity.

Different types of anhydrous lactose powders consist of various ratios of the two optically isomeric forms α- and β-lactose, which will influence their physical and chemical properties (Lerk, 1983; Olano et al., 1983; Bolhuis et al., 1985), e.g. the ability to withstand the incorporation of water of hydration. Roller-dried β-lactose has been investigated in this respect by microcalorimetry (Angberg et al., 1991a). In a continuation study (Angberg et al., 1991b), the same type of lactose was mixed with microcrystalline cellulose to investigate how that addition influenced the hydrate formation rate of the lactose.

Correspondence: M. Angberg, Department of Pharmaceutics, Uppsala Biomedical Centre, Box 580, S-751 23 Uppsala, Sweden.

In those latter studies, the powders were stored for different lengths of time in the range 33–94% RH (relative humidity) before the intermittent microcalorimetric measurements. Due to the fact that the incorporation of water had progressed during storage, information about the reaction was reduced, especially at the beginning but also between the separate microcalorimetric measurements. This is because the microcalorimeter monitors the heat flow, and thus the rate, at that particular moment. It was therefore of interest to design a technique for continuous measurements at a constant relative humidity, which also included the initial reaction. Furthermore, it was desirable to reduce the sample weight necessary for a measurement. This is a valuable feature if the microcalorimetric technique is used in the preformulation stage when only small amounts of a substance might be available.

The possibility of letting the water vapour in from the outside to interact directly with the solid in the microcalorimeter has been investigated by others (Buckton and Beezer, 1988; Buckton et al., 1988; Blair et al., 1990). The principle of having water or a saturated salt solution in a separate container within the microcalorimetric sample vessel to obtain a constant relative humidity, functioning as a miniature humidity chamber, has been used in this study. A similar approach has been reported by Byström (1990) in a study conducted simultaneously with, but independently of the present investigation, by examining the recrystallization of a drug that had attained amorphous regions due to micronization.

The technique involves additional heat contributions apart from the reaction of interest and it cannot be excluded that these are of importance for the total heat recorded by the microcalorimeter. These contributions include, for example, the heat from the vaporization of water and the adsorption of water by the powder.

The object of this study was to design and evaluate a microcalorimetric measurement technique that makes it possible to monitor a process in the solid state continuously, in abundant water vapour, and preferably also to monitor the initial part of the process. The reaction examined was the incorporation of water of hydration in various types of anhydrous lactose powder at 100% RH. Other concurrent processes were defined and their possible heat contributions to the heat monitored were investigated. Other microcalorimetric measurement techniques were designed to perform these investigations.

Materials and Methods

Materials

Lactose N.F. anhydrous direct tableting 59009 (prepared by roller-drying, called roller-dried β-lactose), lot no. 8NH01, was obtained from Sheffield Products, Norwich, NY, U.S.A.; α-lactose anhydrous (prepared by thermal dehydration, designated as α-lactose anhydrous), β-lactose (crystalline, called crystalline β-lactose) and α-lactose monohydrate, Pharmatose® 200 M

TABLE 1

The approximate amounts of anhydrous β-lactose, anhydrous α-lactose and α-lactose monohydrate in percent in the different lactose types studied

	β-lactose, anhydrous (%)	α-lactose, anhydrous (%)	α-lactose, monohydrate (%)
Roller-dried β-lactose	69	22	9
α-Lactose anhydrous	20	71	9
Crystalline β-lactose	94	5	1
Commercial α-lactose monohydrate	4	0	96

The α- and β-lactose ratio was analysed by DMV, The Netherlands, using gas chromatography. The α-lactose monohydrate content was obtained using differential scanning calorimetry.

(referred to as commercial α-lactose monohydrate) were all purchased from De Melkindustrie Veghel bv (DMV), Veghel, The Netherlands.

In Table 1, the approximate amounts of anhydrous β-lactose, anhydrous α-lactose and α-lactose monohydrate in the types of lactose used in this study are listed. These starting materials are sometimes referred to as untreated material in the text, to differentiate them from material that has been stored at specified relative humidities before the microcalorimetric measurements.

Methods

Microcalorimetric measurements

Glass vials Samples of lactose were placed at the bottom of a sample vessel, a 3.2 ml glass vial. A small container (a plastic protective cap for syringes, Viggo AB, Sweden) was filled with approx. 80 μl distilled water and placed within the sample vessel in the powder bed. The vessel was closed with a teflon-lined rubber seal and an aluminium cap. The water vaporized and 100% RH was eventually attained. The sample vessel thus simultaneously became a miniature humidity chamber. The reference vessel was filled with 100 mg commercial α-lactose monohydrate without the addition of the water container. Immediately after closure, the vessels were inserted into the isothermal heat-conduction microcalorimeters (2277 Thermal Activity Monitor; Thermometric AB, Sweden) (Suurkuusk and Wadsö, 1982).

The first 30 min were allowed for temperature equilibration, before the computer's data-collecting program was started (referred to as $t = 0$). The experimental temperature was 25.0°C. The heat flow curves monitored were integrated over time and the cumulative heat was obtained. Exothermic heat flow signals are given positive values in this paper.

Measurements were performed with 100.0 ± 1.0 mg of all lactose types. A 50.0 ± 1.0 mg sample of roller-dried β-lactose was also monitored. Measurements were also performed without powder, but with the water container, to monitor the heat flow of the water vaporization and adsorption to the surfaces of the sample vessel. The precision was evaluated by performing six measurements with 100.0 mg roller-dried β-lactose. The 95% confidence intervals for the cumulative heat and the duration of the process were calculated.

The anhydrous lactose types used were also exposed to water vapour in the range 58–94% RH for 93 days as described earlier (Angberg et al., 1991a). Roller-dried β-lactose was additionally stored for 22 days. After storage, 100.0 ± 1.0 mg of the stored samples were monitored at 100% RH, as described above.

Modified perfusion cell The microcalorimetric system can be utilized for many different types of studies, e.g., perfusion and titration experiments (Suurkuusk and Wadsö, 1982). The so-called perfusion cell, made of stainless steel, consists of a sample vessel and a tube, that connects the sample vessel with the outside. In the tube there is a shaft that can be used for stirring and continuous flow experiments. A titration cannula can be inserted into another inlet. The perfusion cell was modified as described below.

Model 1. To observe the heat flow signal from the start, which otherwise was lost during the 30 min temperature equilibration period, the stirring shaft was removed and the sample vessel was closed at the connection point between the vessel and the tube. The volume was hereby only slightly larger than for a glass vial. The small, empty water container was positioned in the powder sample at the bottom and the titration cannula was fixed within the small container. The perfusion cell was temperature equilibrated for 30 min in the microcalorimeter and then inserted into the measurement position. When a zero signal had been attained, about 80 μl of distilled water was injected into the small container. The water vaporized and the additive heat flow signal from the various processes was monitored.

Model 2. Another type of measurement allowed the water to vaporize from a distant position from the sensitive thermopiles, that is situated around the sample vessel in the measurement position. Not only the stirring shaft, but also the titration cannula were removed during these measurements. The powder sample was placed at the bottom of the sample vessel. The tube that connects the sample vessel with the outside was filled with water so that the water meniscus was approx. 6 cm from the thermopiles and 8 cm from the powder sample. The water vaporized and interacted with the powder, but the heat from the vaporization, which otherwise was included in the ordinary measurements, was hereby excluded.

The two perfusion cell experiments were performed with 100.0 ± 1.0 mg roller-dried β-lactose, both an untreated sample and a sample, stored for 93 days at 94% RH, for which the incorporation process was completed. As a reference, a stainless-steel vessel filled with 100 mg commercial α-lactose monohydrate, was used.

Differential scanning calorimetry (DSC) measurements

DSC measurements were performed to analyse the hydrate content by measuring the heat of dehydration for the various anhydrous lactose types examined, both before and after the microcalorimetric measurements, and to characterize some of the crystallographic changes that appeared. A DSC 20 (Mettler, Switzerland) was utilized as in the previous study (Angberg et al., 1991a). The DSC sample weights were 2.6–3.6 mg. The integration temperature range for the peak of dehydration varied, depending both on the type of lactose used and on the amount of water of hydration. The mean values reported are from at least three measurements. The endothermic heat of dehydration values are given positive values in this paper. The amount of the lactose that is in the form of monohydrate in % of the total lactose content was calculated by dividing the heat of dehydration value obtained by the heat of dehydration value for a sample (of the same lactose type) for which the incorporation process was completed.

Total water uptake measurements

The total water uptake was obtained gravimetrically by accurately weighing powder samples (5 decimal balance) before and after the microcalorimetric measurement. The weight increase was then recorded. These measurements were performed with 50.0 and 100.0 mg samples of untreated roller-dried β-lactose and 100.0 mg roller-dried β-lactose stored for 93 days at 94% RH, i.e., for which the incorporation process was completed.

Interrupted measurements

Roller-dried β-lactose samples of 100.0 ± 1.0 mg were further analysed by investigating the progressive changes during the microcalorimetric measurement. The measurements were interrupted after 0, 1, 2, 3, 4, 5, 6, 8, 12, 18, 24, 48, 72, 96, 120 and 144 h. 0 h represents the time after the temperature equilibration period (30 min). The cumulative heat values for the microcalorimetric measurements were calculated. The total water uptake, the heat of dehydration values and the amount of water of hydration were determined for the powder samples after the microcalorimetric measurements had ended.

Results and Discussion

Measurements in glass vials

Description of the heat flow curves for untreated lactose The heat flow curves for 100.0 mg of the lactose types examined are shown in Fig. 1. The

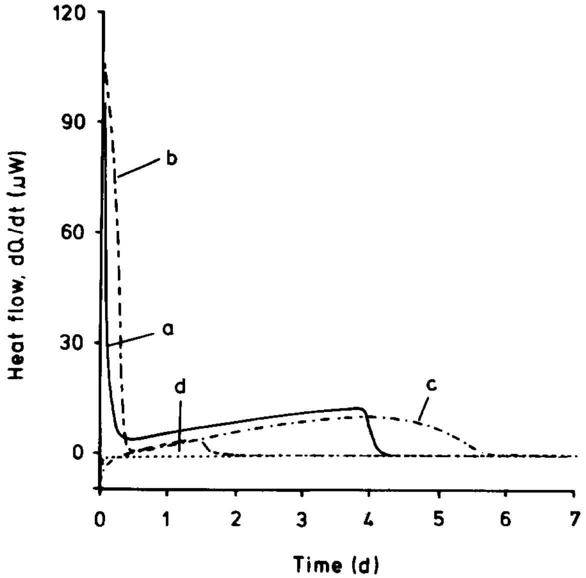

Fig. 1. Heat flow curves for 100.0 mg lactose measured in glass vials at 100% RH. Curves: (a) roller-dried β-lactose (———); (b) (α-lactose anhydrous (- — — —); (c) crystalline β-lactose (· – · – · –) and (d) α-lactose monohydrate (· · · · · ·).

amounts of anhydrous α- and β-lactose are noted in brackets and in Table 1. The curve (Fig. 1a) for roller-dried β-lactose (22% α, 69% β) starts with a high value which descends very rapidly to a minimum value which is reached after about 8 h. After that, the signal increases and then, after nearly 4 days measurement time, falls to zero over a period of about 10 h. The curve (Fig. 1b) for α-lactose anhydrous (71% α, 20% β) also starts with a high heat flow which decreases to nearly zero after about 10 h. It then increases and after less than 2 days measurement, it falls to zero. The curve (Fig. 1c) for crystalline β-lactose (5% α, 94% β) starts with an endothermic signal, crosses zero after about 10 h, increases slowly and falls to zero after about 6 days. The curve (Fig. 1d) for commercial α-lactose monohydrate (0% α, 4% β) shows that a powder that is already in the form of monohydrate has almost no signal and that the heat flow contributions from, e.g., vaporization and adsorption, are negligible. These facts support the suggestion that the heat flow signals for the curves Fig. 1a–c come mainly from the incorporation of water of hydration.

The curves Fig. 1a–c seem to be divided into two parts, proportional to the amounts of anhydrous α- and β-lactose in the samples. The more anhydrous α-lactose, the larger the area under the curve in the first part and the more anhydrous β-lactose, the larger the area in the second part. The incorporation of water of hydration into roller-dried β-lactose was described by Angberg et al. (1991a). Biphasic behaviour was demonstrated, especially at the highest humidity investigated, 94% RH. The first phase represented incorporation of water, mainly in original anhydrous α-lactose. This seems to correspond to the first part of the curves in Fig. 1. For roller-dried β-lactose (Fig. 1a) and α-lactose anhydrous (Fig. 1b), this part slopes steeply. This indicates that the rate of the incorporation process decreases rapidly, due to the decreasing amount of original anhydrous α-lactose left with which to react. For crystalline β-lactose (Fig. 1c), no first part is visible, due to the small content of anhydrous α-lactose.

The second phase in the biphasic process described by Angberg et al. (1991a) represented incorporation mainly in anhydrous α-lactose transformed from mutarotated β-lactose, and this seems to correspond to the second part of the curves in Fig. 1. The mutarotation was shown to be a rate-limiting step. It also seems to be rate-limiting in this study at 100% RH, as the second part shows that the heat flow signal continues at a low level for a long time. The second part even ascends, which means either that the rate of the incorporation process actually increases with time or that there are other heat flow contributions that influence the monitored heat flow curves at this high humidity. In the previous study, where the highest humidity during storage was 94% RH, a decrease of the heat flow level was seen in the second phase.

It was shown by Angberg et al. (1991a) that the incorporation in anhydrous α-lactose that has mutarotated from β-lactose starts as a parallel process before the incorporation in original anhydrous α-lactose has ended, which is also relevant in this study. The incorporation process is therefore not definitely divided into two parts, but as a basis for the discussion it is a useful simplification.

Interrupted measurements In a series of microcalorimetric measurements, the incorporation process in roller-dried β-lactose was interrupted and the cumulative heat, the water uptake and the heat of dehydration were determined for the different time intervals.

The cumulative heat from the microcalorimetric measurements and the heat of dehydration values from the DSC measurements are normalized in Fig. 2. If the heat flow curve (Fig. 1a) corresponds entirely to the incorporation process, the two curves in Fig. 2 ought to have the same shape. As can be seen, they have similar appearances except for the first 24 h, when the cumulative heat curve shows a higher level than the dehydration curve. After 96 h (4 days), plateaus are reached for both curves. The heat of dehydration is endothermic and is caused by the breaking of the bonds between the water of hydration and lactose and additionally by the vaporization of that water, calculated per g of lactose. The plateau ends at 181 J/g. The measurements after 6 days (144 h) had a 95% confidence interval of 178–184

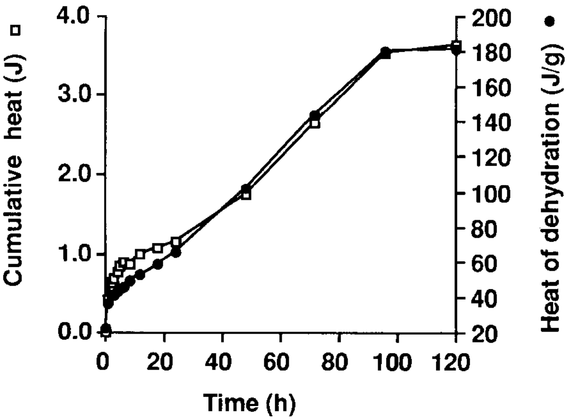

Fig. 2. The cumulative exothermic heat obtained by microcalorimetry (□) and the endothermic heat of dehydration values obtained by DSC (●) as a function of time for 100 mg roller-dried β-lactose after interrupted measurements in glass vials at 100% RH. The heat of dehydration at $t = 0$ is 22 J/g.

J/g ($n = 10$). This value corresponds, according to these DSC measurements, to roller-dried β-lactose that has been totally transformed to α-lactose monohydrate.

The precision of the microcalorimetric technique was investigated by repeating six similar measurements. The mean value for the cumulative heat was 3.55 J and the 95% confidence interval was 3.48–3.61 J. The mean duration of the incorporation process was 97 h, with the 95% confidence interval 95–100 h. The reproducibility must partly depend on the inherent variability in the amount of anhydrous α- and β-lactose in each sample. The cumulative heat for the measurement presented in Fig. 2 ends at an exothermic value of 3.6 J. The heat obtained is the additive heat of all processes, both exothermic and endothermic, that have proceeded in the sample vessel, including the bonding between water of hydration and lactose, i.e., the incorporation process.

As there is no substantial heat flow after the process has ended after 96 h (Fig. 1a), there is no dissolution of the lactose, provided that neither the heat of dissolution nor the amount dissolved is very small. Dissolution of lactose in water is an endothermic process. Hudson and Brown (1908) reported a value of 9.6 J/g for what they called the initial heat of solution of anhydrous β-lactose and 50.2 J/g for α-lactose monohydrate. Fielden et al. (1988) reported a heat of dissolution for commercial α-lactose monohydrate of 59.5 J/g. The curve for commercial α-lactose monohydrate (Fig. 1d) showed an endothermic heat flow of around 1 μW, which can indicate a minute dissolution process. 1 μW for 97 h (the mean duration of the incorporation process) corresponds approximately to the dissolution of 6% of the sample.

The second part of the curve of Fig. 1a and the corresponding cumulative curve in Fig. 2 indicate an increased incorporation rate with time. This is not contradicted by the heat of dehydration curve in Fig. 2, as this curve also shows positive deviation from a straight line. However, the deviation is not very pronounced and the possible increased rate will therefore be discussed further below.

Roller-dried β-lactose transformed totally to α-lactose monohydrate gave a mean heat of dehydration value of 181 J/g, as mentioned above. This in turn contains 5% water, as calculated from the molecular weights of water and α-lactose monohydrate (18/360). It was also possible to calculate the % water incorporated at intermediate stages and these values are compared to the total water uptake in Fig. 3. As can be seen, the total water uptake represents a much larger weight than the incorporated water for each time interval and when the plateau is reached after 96 h (4 days), i.e., the incorporation reaction is completed, the uptake of water still continues.

The small container in the glass vial is filled with a surplus of water and hence the process proceeds in abundant water vapour. However, the water vapour is not instantaneously available for the incorporation process, since it must first vaporize from the water surface. In general terms, the extent of the water vaporization in a closed container at constant temperature depends on the ability of the powder to take up/consume water, e.g., adsorption, absorption, formation of water of hydration and other chemical reactions, as this amount must be compensated for to maintain the equilibrium relative humidity.

The total water uptake measured after 96 h was 13.8% w/w for the untreated roller-dried β-lactose and 6.9% w/w for the powder in which

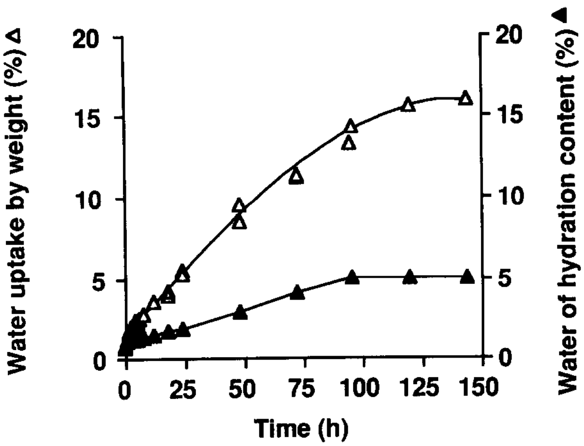

Fig. 3. Total water uptake by weight in % (△) and the calculated water content in the form of water of hydration in % (▲) as a function of time for 100 mg roller-dried β-lactose after interrupted measurements in glass vials at 100% RH. The amount of water of hydration in the untreated roller-dried β-lactose is 0.47% and this value has also been added to the total water uptake values.

the incorporation reaction was completed. This shows that the rate of water uptake is lower for a sample that is already hydrated. The effect of the sample size was also investigated. The incorporation process for a 50.0 mg sample of roller-dried β-lactose continued for approx. 68 h and the total water uptake was 19.0% w/w. Firstly, this shows that it takes more than half the time to transform half the sample weight to the monohydrate and secondly that the total water uptake in % w/w is much larger for half the sample weight. However, if the absolute uptake of water is compared instead, the amount is 9.5 mg/50 mg powder and 13.8 mg/100 mg powder. Finally, if the absolute amount taken up is divided by the duration of the incorporation process, the value obtained is approx. 0.15 mg water taken up per h for both sample sizes. However, as can be seen in Fig. 3 for a 100 mg sample, the water uptake is not linear with time.

The large amount of water taken up by the powder at 100% RH is probably because it is above the critical relative humidity, RH_0, for roller-dried β-lactose. The RH_0 is characteristic of the solid and above this humidity the adsorbed water assumes the character of bulk solution. The water condensed on the surface will start to dissolve the solid and saturate the liquid film. A saturation level for the amount of water taken up will not be obtained until the lactose has totally dissolved (Van Campen et al., 1983).

Measurements in the perfusion cell

The initial reaction period A disadvantage with the microcalorimetric measurements performed in glass vials is that the initial reaction during the 30 min temperature equilibration period cannot be followed. To monitor the absolute start, the modified perfusion cell (model 1) was used, which means that the small container is filled with water *after* the temperature equilibration period. This also means that the initial heat flow from the vaporization of water can be monitored, until the heat flow from the adsorption of the water vapour cancel out this effect. This situation prevails when the heat of adsorption is equal to the heat of condensation, and thus has the same size, but reversed sign, as the heat of vaporization, i.e. 44 kJ/mol at 25°C (Mörtstedt and Hellsten, 1985).

The heat flow signal for 100.0 mg of untreated roller-dried β-lactose started with a minute endothermic signal. However, almost directly it was exceeded by an exothermic heat flow due to the incorporation process. The heat flow signal increased and after about 30 min the maximum heat flow signal was monitored. The heat flow seen at $t = 0$ for the measurement in the glass vial (Fig. 1a) is therefore very close to the real maximum. The rest of the signal had the same appearance as in the glass vial, which showed that the stainless-steel surfaces and the somewhat larger volume did not influence the measurement. The cumulative heat obtained during the ordinary temperature equilibration period was approx. 0.15 J.

The same type of measurement was performed with roller-dried β-lactose for which the incorporation process was completed. The endothermic heat was much larger than for the untreated powder, but still small, less than 0.1 J. This is a representation of the initial heat of vaporization before the heat of adsorption has cancelled out this effect, because there was no heat from any incorporation of water. The heat flow signal

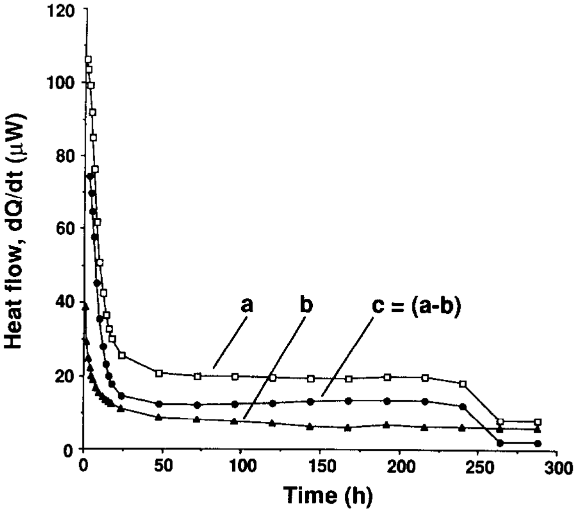

Fig. 4. Values obtained from the heat flow curves for 100.0 mg roller-dried β-lactose measured in the perfusion cell (model 2). Curve (a) used an untreated sample (□) and curve (b) used a sample for which the incorporation reaction was completed (▲). In curve (c) (●) the values from curve (b) have been subtracted from curve (a). See the text for further information.

showed a continuous endothermic heat flow of approx. 1 μW after only 1.5 h. The heat flow contributions in an ordinary sample vessel without powder were compared with the result above. An endothermic heat of less than 0.03 J was monitored in the first hour.

Measurements without influence from the heat of vaporization In order to investigate if the increase in the heat flow shown in the second part of the curve of Fig. 1a really represents an increased incorporation rate, the modified perfusion cell (model 2) was used. This model excludes the heat from the vaporization of water from the monitored heat flow, which means that the heat contribution from the adsorption process also can be studied. Heat flow values were picked from the original heat flow curves and plotted as shown in Fig. 4. Fig. 4a represents untreated roller-dried β-lactose. It shows a rapid decline in the first part of the curve while the second part runs parallel to the baseline. The following observations were made. The heat produced is much larger than seen in the curve of Fig. 1a. This is an indication that the endothermic heat of vaporization is excluded totally or to a great extent. A minimum heat flow value is not obtained between the two parts. The second part indicates a zero-order incorporation process, as it is parallel to the baseline. The whole process takes 264 h (11 days) to finish, which is nearly 3 times as long as in the glass vials. Instead of falling to zero after the second part has ended, the curve shows a consistent heat flow level, due to the continuous adsorption of water.

The curve of Fig. 4b represents roller-dried β-lactose, for which the incorporation reaction is completed, and the heat flow is therefore only due to the adsorption of water. The heat flow is exothermic and largest in the first part, but not as large as for the untreated powder. The second part shows a decreasing heat flow signal, which after 11 days is on a somewhat lower level than for the untreated sample. The first part shows either that there is rapid adsorption of a large number of water molecules or that the heat of adsorption is larger for the first molecules adsorbed. However, this latter explanation would result, for the measurements in the glass vial also, in an exothermic heat flow at the beginning of the curve of Fig. 1d for the commercial α-lactose monohydrate. This is not seen and the larger heat flow in the first part is therefore due to the rapid adsorption of a large number of water molecules, which is otherwise directly compensated for by a similar heat flow from the vaporization.

By subtracting the values in the curve of Fig. 4b from the curve of Fig. 4a, the increased incorporation rate in the second part could possibly be investigated. These values are shown in Fig. 4c and it will ideally not have any contributions from either the heat of vaporization or adsorption. It resembles the heat flow signal for the curve of Fig. 1a, but it takes a longer time for the incorporation process to finish and the increase of the heat flow signal is not at all as pronounced in the second part. In fact, with the constructed curve, a near zero-order incorporation rate was obtained.

In general terms, some factors that influence the rate of attaining equilibrium for the water vapour include the types of water container and sample vessel used, e.g. the water surface area, the gas volume and whether the water vapour has a free passage to penetrate quickly to all spaces in the vessel. These factors are especially important for the passive transport of water molecules. A microcalorimetric system that could be evacuated, as described by Buckton and Beezer (1988), would not have this type of problem. When the vacuum is released in such a system, there is an almost instantaneous transport of water vapour to the powder sample. In the perfusion cell (model 2) the inner diameter of the tube is 4 mm and the water molecules have to pass about 3 cm before reaching the larger sample vessel. This obstructs the transport of water vapour. The total water taken up by the untreated sample (Fig. 4a) was 6.1% w/w, while 3.4% w/w was taken up for the completely hydrated sample (Fig. 4b) after 11 days. This is less than half of the amount measured in the glass vials after 4 days (96 h).

The difference in the amount of water taken up by the samples investigated in the perfusion cell shows again that the water uptake rate was higher for an untreated sample (6.1% w/w), compared to a sample for which the incorporation reaction was completed (3.4% w/w). This is also demonstrated by the fact that the heat flow level for the curve of Fig. 4b does not end at the same heat flow level as the curve of Fig. 4a. This will also influence the curve of Fig. 4c, as this curve would actually have a lower heat flow level if this fact was compensated for. Since water vapour transport in the perfusion cell is not ideal, the weak heat flow increase for the curve of Fig. 4c cannot be used to prove an increased rate in the second part of the incorporation for measurements performed in glass vials, as indicated in Fig. 1.

Contributions to the monitored heat for roller-dried β-lactose

The mutarotation process If the assumption, that the second part of the curve of Fig. 1a corresponds to an increased incorporation rate, is correct for a measurement in the glass vial, the rate of the mutarotation from β- to α-lactose must also be increased, because this is the rate-limiting step. A possible explanation for this increased mutarotation rate could be that the rapid increase in the concentration of adsorbed water (Fig. 3) facilitates the mutarotation process. This is further supported by comparison with the perfusion cell measurement (model 2) (Fig. 4). The uptake of water in this model was markedly less, so that the mutarotation rate was decreased and the duration of the process was extended considerably.

For the incorporation process to be completed, the entire β-lactose content must mutarotate to α-lactose. A value of 1 cal/g was reported as the energy required for the transformation from β- to α-lactose by Hudson and Brown (1908). This corresponds to 0.3 J, if 69% β-lactose of the 100 mg untreated roller-dried β-lactose sample is transformed to α-lactose.

The heat of mutarotation may account for the difference in the curve shapes in Fig. 2. The exothermic heat flow from the incorporation process is reduced by the endothermic heat flow from the mutarotation process. To compensate for the mutarotation, the cumulative heat curve in Fig. 2 must be increased by 0.3 J. However, it is only the second part of the curve that must be increased, because it is here that the mutarotation proceeds. Furthermore, the 0.3 J cannot be added evenly to the cumulative curve, instead it must increase as a percentage of the monitored heat. If this constructed cumulative heat curve is normalized with the heat of dehydration curve, they will be more similar in shape than shown in Fig. 2. The influence of the mutarotation is also shown for crystalline β-lactose (Fig. 1c). It even has a pronounced endothermic start, because the mutarotation reduces the heat flow below zero.

As discussed earlier, the incorporation process for a 100.0 mg sample of roller-dried β-lactose continued for 97 h, while that for a 50.0 mg sample took 68 h, which means that it takes more than half the time to finish the process for half the sample weight. The appearance of the second part of the heat flow curves was almost identical between 10 and 60 h, which means that the same amount of water gets incorporated during this period independently of the sample weight. This can be explained by the fact that the absolute amount of water taken up is approximately the same, about 10 mg, during the first 68 h for both sample weights. This amount can transform the same amount of anhydrous β-lactose to α-lactose monohydrate.

The cumulative heat for the 50 mg sample was 1.6 J, which is less than half the value obtained for a 100 mg sample, 3.6 J. This can at least partly be explained by the fact that a larger fraction of the incorporation process in original anhydrous α-lactose has proceeded for the 50 mg sample during the 30 min temperature equilibration period, which is not monitored by the microcalorimeter.

The heat evolved due to the incorporation of water The various types of heat contributions that are involved in the incorporation process for 100.0 mg roller-dried β-lactose, obtained by microcalorimetry, have been summarized in Table 2 from the result presented above. The heat obtained from vaporization and adsorption cancel each other out almost entirely. The mean cumulative heat monitored by the microcalorimeter is 3.55 J. This value is increased by adding 0.15 J for the heat lost during the 30 min equilibration period. The heat from the incorporation process is reduced by 0.3 J due to the mutarotation process and 0.3 J due to a possible dissolution, which must also be added. This gives a total heat of 4.3 J. This value corresponds approximately to the incorporation of 2.66×10^{-4} mol water, since 9% of the untreated lactose was in the form of the monohydrate (Table 1). This gives an exothermic enthalpy change of the incorporation process, the bonding of water to roller-dried β-lactose, of 16 kJ/mol. This can be compared to the bond energy range for hydrogen bonds of 8–33 kJ/mol (Martin et al., 1983), since this is the most likely bond involved (Berlin et al., 1971).

The maximum heat of dehydration value obtained by DSC is 181 J/g, with the peak temperature around 135°C. This gives an endothermic enthalpy change of 65 kJ/mol (181 J/g × 360 g/mol). As this includes the enthalpy of vaporization of 39 kJ/mol at 135°C (Mörtstedt and Hellsten, 1985), the resulting enthalpy change is 26 kJ/mol for the breaking of the hydrogen bonds between water and lactose. The reason for the discrepancy with the value obtained by microcalorimetry (16 kJ/mol) is probably that the obtained value (181 J/g) is too high. This may be due to how the integration of the peak area is calculated, because a DSC measurement for commercial α-lactose monohydrate with the same integration programme gave a value of 157 J/g (Angberg et al., 1991a), which is higher than the heat of dehydration reported earlier for commercial α-lactose monohydrate, 144 J/g (52 kJ/mol) (Berlin et al., 1971; Vromans et al., 1985). It therefore appears that the value of 181 J/g should also be somewhat reduced, which makes the enthalpy change obtained by DSC similar in size to that obtained by microcalorimetry.

It must be concluded that even if there are other heat flow contributions to the curves in Fig. 1, they give a characterization of the incorporation process and that the technique used here can

TABLE 2

The various heat contributions for 100.0 mg roller-dried β-lactose (with the composition presented in Table 1) when it is converted to α-lactose monohydrate

Heat contributions	Exothermic/ endothermic	Size (J)	Comments
Vaporization	endothermic	34 [a]	Cancel each other out
Adsorption/(condensation)	exothermic	34 [a]	
Dissolution	endothermic	0.3	
Mutarotation	endothermic	0.3 [b]	
Hydrogen-bonding [c]	exothermic	4.3	

[a] Calculated from the amount of the total water taken up after 96 h (Fig. 3) and the heat of vaporization at 25°C, 44 kJ/mol (Mörtstedt and Hellsten, 1985).

[b] From Hudson and Brown (1908).

[c] The bonding type most likely involved between water of hydration and lactose (Berlin et al., 1971).

be utilized to compare the sensitivity to water of the various anhydrous lactose types examined.

Powders prestored at various RH before measurement at 100% RH

Anhydrous lactose powders, which have been exposed to water vapour beforehand, have various amounts of hydrate water incorporated depending on the humidity and the duration of this exposure. This will influence the appearance of the heat flow curves, shown below, when later measured at 100% RH, compared to a heat flow curve for an untreated sample. This means that microcalorimetry may be useful in the control of raw materials, to investigate if a powder has been exposed to water vapour.

Heat flow curves obtained from *roller-dried β-lactose* samples, which had been stored for 22 days in the range 58–94% RH, and thereafter measured in the microcalorimeter at 100% RH, are shown in Fig. 5a. A sample stored at 58% RH has a similar curve to that of an untreated powder (Fig. 1a). Samples stored at 75, 81 and 84% still retain a small segment of the first part, but for powders stored at 94% RH, that part is lost. In such a powder probably all the original anhydrous α-lactose has already reacted. As can be seen at the end of the second part of the curve, the incorporation reaction ceases earlier for the sample that has been stored at the highest humidity beforehand, obviously because there is a smaller amount of anhydrous lactose to react with.

In Fig. 5b, the roller-dried β-lactose powders had been stored for 93 days before the measurements at 100% RH. The heat flow curve for a powder stored at 58% RH even had a longer duration than the upper limit of the 95% confidence interval for an untreated powder. Powders stored at 75 and 81% RH retain some of the first part of the curve, but for a powder stored at 84% RH, that part has disappeared. The termination of the incorporation process is even more separated than after 22 days. For powders stored at

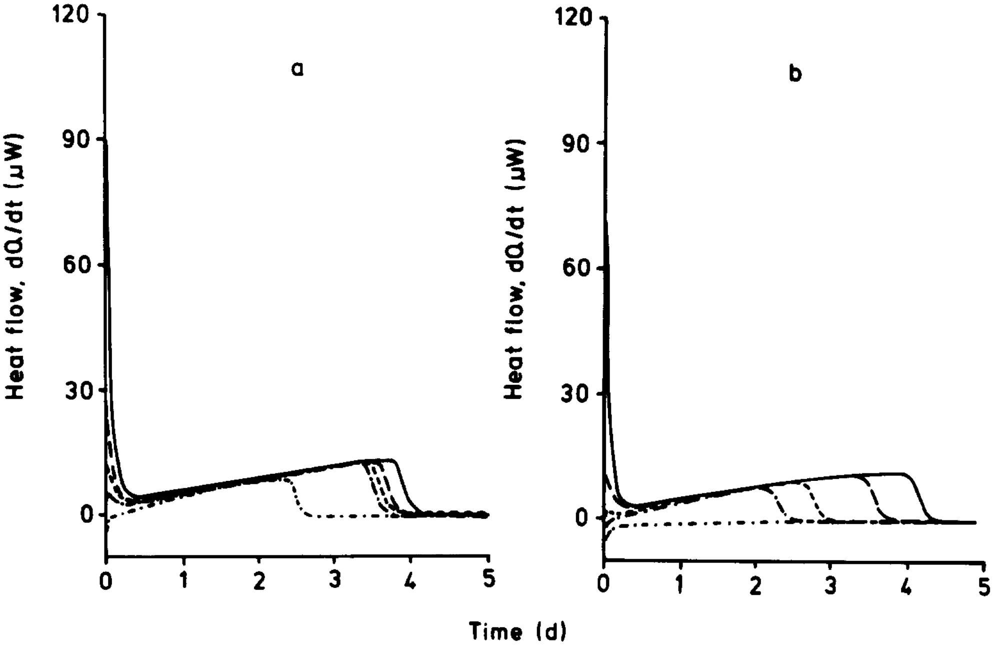

Fig. 5. Heat flow curves for 100.0 mg roller-dried β-lactose measured in glass vials at 100% RH after storage for (a) 22 days and (b) 93 days at 58% (———), 75% (— — —), 81% (- - - - - -), 84% (- —— - —— -) and 94% (–·–·–·) RH.

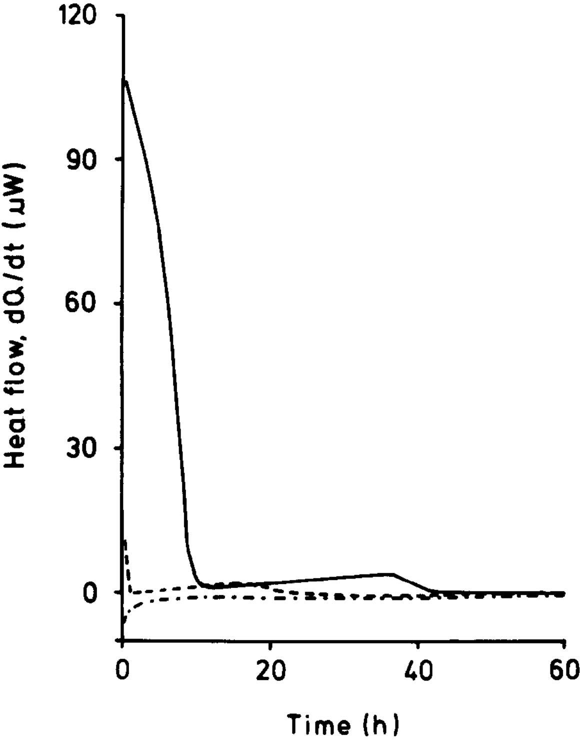

Fig. 6. Heat flow curves for 100.0 mg α-lactose anhydrous measured in glass vials at 100% RH after storage for 93 days at 58% (———), 75% (- - - - - -) and 84% (–·–·–·) RH.

94% RH, there is almost no heat flow signal, because the incorporation process was already completed in these samples. The curves for these powders, as was also the case for the curves for commercial α-lactose monohydrate, had an endothermic start that was due to the vaporization of water and a continuous endothermic heat flow of approx. 1 μW, probably due to dissolution.

Fig. 6 shows heat flow curves obtained at 100% RH for *α-lactose anhydrous* powders that have been stored for 93 days at 58, 75 and 84% RH. Powders stored at 58% seem to be unchanged compared to the untreated material (Fig. 1b). Powders stored at higher humidities showed large changes. At 75% RH, only a small segment of the first part of the curve is left, which was the same for a powder stored at 81% RH. At 84% RH, the incorporation processes seem to have ended, which was also the case for a powder stored at 94% RH.

Crystalline β-lactose stored for 93 days at 58, 75, 81 or 84% RH had approximately the same heat flow curves at 100% RH (not shown) as untreated powder (Fig. 1c). For powders stored at 94% the heat flow signal decreased to zero, i.e., the incorporation process was completed, approx. 70 h before the other samples stored at the lower humidities. Crystalline β-lactose is a type of anhydrous lactose which is undoubtedly much more stable against incorporation of water, due to the rate-limiting mutarotation. This is also seen at 100% RH in Fig. 1, as the incorporation reaction proceeds for 6 days (Fig. 1c) compared to less than 2 days for α-lactose anhydrous (Fig. 1b).

Table 3 shows the heat of dehydration values and the hydrate content obtained for the samples investigated after storage at lower humidities and thereafter used in the microcalorimetric measurements at 100% RH. Heat of dehydration values for the untreated material and after the incorporation process has ended after measurement at 100% RH for the samples shown in Fig. 1a–c, are included. It can be observed that the maximum heat of dehydration values are different for the anhydrous lactose types investigated and also that they are different from commercial α-lactose monohydrate which had a value of 157 J/g, obtained by the same integration technique (Angberg et al., 1991a). The difference in the heat of dehydration values is only 6 J/g for untreated roller-dried β-lactose and a powder that has been stored at 58% RH for 93 days, which explains the similarity of the heat flow curves.

In Fig. 7a, the DSC scans for α-lactose anhydrous are shown after storage for 93 days at 58–94% RH. The dehydration peaks change in appearance in a way not seen for roller-dried β-lactose. DSC scans for roller-dried β-lactose have been presented earlier (Angberg et al., 1991a). The heat of dehydration for a powder stored at 84% RH even had a lower value than for those stored at 75 and 81% RH (Table 3). Furthermore, the dehydration peaks for powders stored at 84 and 94% RH were similar neither in appearance (Fig. 7a) nor in size (Table 3), but the heat flow curves indicated that the incorporation processes were completed for both samples. Ad-

TABLE 3

The heat of dehydration and the monohydrate content in % of the maximum value obtained for roller-dried β-lactose, α-lactose anhydrous and crystalline β-lactose for the untreated powders and for powders that have been stored in humidity chambers at various relative humidities for various lengths of time

Relative humidity (%)	Time (days)	Roller-dried β-lactose		α-Lactose anhydrous		Crystalline β-lactose	
		Heat of dehydration (J/g)	Monohydrate content (%)	Heat of dehydration (J/g)	Monohydrate content (%)	Heat of dehydration (J/g)	Monohydrate content (%)
Untreated	0	17	9	15	9	2	1
58	22	19	10	–	–	–	–
75	22	45	25	–	–	–	–
81	22	58	32	–	–	–	–
84	22	63	35	–	–	–	–
94	22	110	61	–	–	–	–
58	93	23	13	35	21	2	1
75	93	71	39	150	90	12	8
81	93	98	54	159	95	18	12
84	93	116	64	144	86	28	18
94	93	181	100	160	96	99	64
100	5	181	100	–	–	–	–
100	2	–	–	167	100	–	–
100	7	–	–	–	–	155	100

The values obtained at 100% RH correspond to the completed incorporation process measured after the microcalorimetric measurements shown in Fig. 1a–c.

ditionally, if the incorporation process had been completed for these samples, the hydrate content should have been 100% (Table 3).

Another observation is that the α-lactose anhydrous powders that have been stored at 75 and 81% RH (Fig. 7a) show an increase of the β-lactose peaks, even though the original β-lactose cannot incorporate water of hydration. This indicates that α-lactose anhydrous passes through an intermediate β-lactose form in the hydration process. All these observations show that the hydrate formation process for α-lactose anhydrous is extremely complicated.

In Fig. 7b, the DSC scans for crystalline β-lactose are shown after storage for 93 days at 58, 84 and 94% RH. The DSC scan for the sample obtained after the microcalorimetric measurement at 100% RH has also been included to show the DSC scan for a powder with a completed incorporation. The dehydration peaks change little in size between 58 and 84% RH, but there is a change in the appearance of the scans. The sample stored at 94% RH changes considerably, which is consistent with the microcalorimetric heat flow result.

Conclusions

Continuous microcalorimetric measurements for solid samples at 100% RH can be performed with a newly designed measurement technique. With this technique, a process can be monitored over a long period of time, because the water vapour is abundant. Furthermore, registration can start very close to the initiation of the process and only small sample sizes are needed. Reproducibility is good, as the measurements are not influenced by transfer of the sample at ambient atmosphere.

The technique can be used for the quality control of powders. The monitored signal at 100% RH for a powder that has been exposed to water vapour will not have the same appearance as that

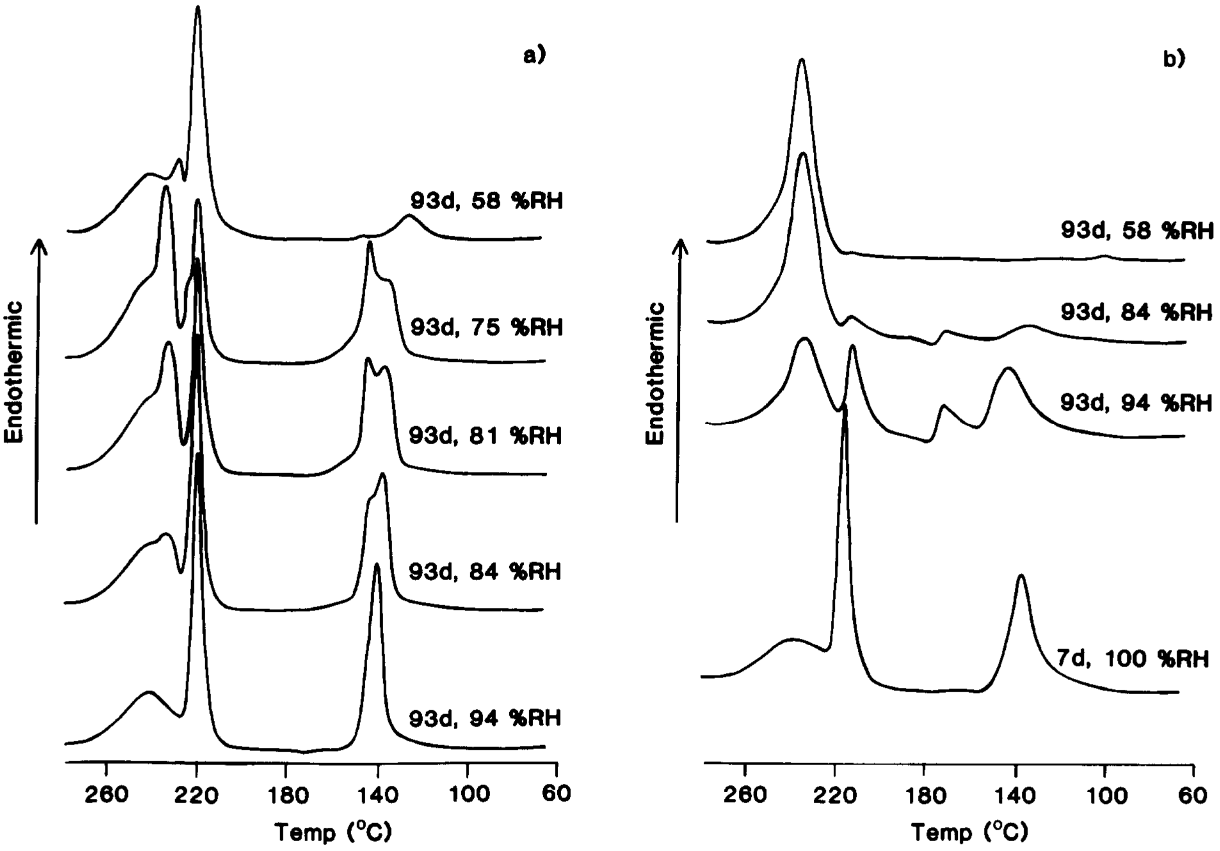

Fig. 7. DSC scans for (a) α-lactose anhydrous after storage for 93 days at 58, 75, 81, 84 and 94% RH and for (b) crystalline β-lactose after storage for 93 days at 58, 84, 94% RH and after 7 days microcalorimetric measurement at 100% RH. The dehydration peak is around 140°C, the fusion of α-lactose around 220°C and the fusion of β-lactose around 235°C. The broad, low peak which is seen clearly or partly behind the β-lactose peak at the end of the investigated temperature interval, represents charring and decomposition of the lactose (Berlin et al., 1971).

of a powder which has not reacted with water.

The monitored heat flow curves give an overall characterization of the incorporation process of water of hydration into anhydrous α- and β-lactose, because the heat flow signal is almost entirely due to the hydrate formation. The enthalpy change for the bonding of water of hydration in roller-dried β-lactose was calculated as 16 kJ/mol.

Acknowledgements

The authors are very grateful to Dr Kussendrager, DMV, The Netherlands, for the generous gift of α-lactose anhydrous and crystalline β-lactose, and information regarding these materials. We also wish to thank Montoil AB, Sweden, for the generous gift of roller-dried β-lactose.

References

Angberg, M., Nyström, C. and Castensson, S., Evaluation of heat-conduction microcalorimetry in pharmaceutical stability studies. I. Precision and accuracy for static experiments in glass vials. *Acta Pharm. Suec.*, 25 (1988) 307–320.

Angberg, M., Nyström, C. and Castensson, S., Evaluation of heat-conduction microcalorimetry in pharmaceutical stability studies. II. Methods to evaluate the microcalorimetric response. *Int. J. Pharm.*, 61 (1990) 67–77.

Angberg, M., Nyström, C. and Castensson, S., Evaluation of heat-conduction microcalorimetry in pharmaceutical stability studies. III. Crystallographic changes due to water vapour uptake in anhydrous lactose powder. *Int. J. Pharm.*, 73 (1991a) 209–220.

Angberg, M., Nyström, C. and Castensson, S., Evaluation of heat-conduction microcalorimetry in pharmaceutical stability studies. IV. The influence of microcrystalline cellulose on the hydration rate of anhydrous lactose. *Int. J. Pharm.*, 77 (1991b) 269–277.

Berlin, E., Kliman, P.G., Anderson, B.A. and Pallansch, M.J., Calorimetric measurements of the heat of desorption of water vapor from amorphous and crystalline lactose. *Thermochim. Acta*, 2 (1971) 143–152.

Blair, T.C., Buckton, G., Beezer, A.E. and Bloomfield, S.F., The interaction of various types of microcrystalline cellulose and starch with water. *Int. J. Pharm.*, 63 (1990) 251–257.

Bolhuis, G.K., Reichman, G., Lerk, C.F., Van Kamp, H.V. and Zuurman, K., Evaluation of anhydrous α-lactose, a new excipient in direct compression. *Drug Dev. Ind. Pharm.*, 11 (1985) 1657–1681.

Buckton, G. and Beezer, A.E., A microcalorimetric study of powder surface energetics. *Int. J. Pharm.*, 41 (1988) 139–145.

Buckton, G., Choularton, A., Beezer, A.E. and Chatham, S.M., The effect of the comminution technique on the surface energy of a powder. *Int. J. Pharm.*, 47 (1988) 121–128.

Byström, K., Microcalorimetric testing of physical stability of drugs in the solid state. *Thermometric Application Note 22004*, Thermometric AB, Järfälla, Sweden, 1990.

Fielden, K.E., Newton, J.M., O'Brien, P. and Rowe, R.C., Thermal studies on the interaction of water and microcrystalline cellulose. *J. Pharm. Pharmacol.*, 40 (1988) 674–678.

Hansen, L.D., Lewis, E.A., Eatough, D.J., Bergstrom, R.G. and DeGraft-Johnson, D., Kinetics of drug decomposition by heat conduction calorimetry. *Pharm. Res.*, 6 (1989) 20–27.

Hudson, C.S. and Brown, F.C., The heats of solution of the three forms of milk-sugar. *J. Am. Chem. Soc.*, 30 (1908) 960–971.

Lerk, C.F., Physico-pharmaceutical properties of lactose. *Proceedings of the 22nd International Colloquium on Industrial Pharmacy, Ghent*, 1983, pp. 59–89.

Martin, A., Swarbrick, J. and Cammarata, A., *Physical Pharmacy*, 3rd Edn, Lea and Febiger, Philadelphia, 1983, p. 60.

Mörtstedt, S.E. and Hellsten, G., *Data och Diagram*, 6th Edn, Esselte Studium, Stockholm, 1985, pp. 39–41.

Olano, A., Corzo, N. and Martinez-Castro, I., Studies on β-lactose crystallization. *Milchwissenschaft*, 38 (1983) 471–474.

Oliyai, R. and Lindenbaum, S., Stability testing of pharmaceuticals by isothermal heat conduction calorimetry: Ampicillin in aqueous solution. *Int. J. Pharm.*, 73 (1991) 33–36.

Pikal, M.J. and Dellerman, K.M., Stability testing of pharmaceuticals by high-sensitivity isothermal calorimetry at 25°C: cephalosporins in the solid and aqueous solution states. *Int. J. Pharm.*, 50 (1989) 233–252.

Suurkuusk, J. and Wadsö, I., A multichannel microcalorimetry system. *Chem. Scr.*, 20 (1982) 155–163.

Van Campen, L., Amidon, G.L. and Zografi, G., Moisture sorption kinetics for water-soluble substances. I: theoretical considerations of heat transport control. *J. Pharm. Sci.*, 72 (1983) 1381–1388.

Vromans, H., de Boer, A.H., Bolhuis, G.K., Lerk, C.F. and Kussendrager, K.D., Studies on the tabletting properties of lactose. Part I. The effect of the particle size on binding properties and dehydration characteristics of lactose. *Acta Pharm. Suec.*, 22 (1985) 163–172.